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## PATENT SPECIFICATION

755,900

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### COMPLETE SPECIFICATION.

#### Improvements in or relating to Photometric Apparatus.

We, NATIONAL RESEARCH DEVELOPMENT CORPORATION, a British Corporation established by Statute, of 1 Tilney Street, London, W.1, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to photometric apparatus, more particularly to nephelometers or turbidometers for use in measuring the concentration of suspensions of particles in a liquid medium.

The object of the invention is to provide an improved apparatus for use with suspensions having a wide range of concentrations and more especially for use with bacterial suspensions which may be of any concentration normally encountered, and to permit the given concentration to be measured directly without the necessity of tedious and possibly hazardous preparations of suitable dilutions.

The apparatus is of the kind in which a beam of light is passed through a cell of suitable thickness containing the suspension. During the passage of the light through the suspension some of the light is scattered by the suspended particles. In a bacterial suspension, the particles size is such that most of this light is scattered in a direction close to that of the primary beam. It can be shown that the ratio of the intensity of the forwardly scattered light to the intensity of the transmitted light is approximately proportional to the concentration of particles in the suspension. By a comparison of the light forwardly scattered by a suspension with the light simultaneously transmitted by the same, it is possible to obtain a measure of the concentration over a wide range.

According to the present invention apparatus for measuring the concentration of a suspension of particles in a liquid medium

by comparing the light scattered by the suspension with the light transmitted by the same comprises a light source a cell for containing the suspension, a lens system for focusing the transmitted emergent light, means associated with the said lens system adapted to be adjusted at will either to cut off the directly transmitted emergent light and to collect the forwardly scattered light from the suspension and direct the same on to a photo-electric device, or to cut off the forwardly scattered light and to direct the said transmitted light on to the said photo-electric device whereby the ratio of the forwardly scattered light from the said suspension to the directly transmitted light and hence the concentration of the suspension can be determined.

Preferably a second photo-electric device is associated with the same light source for balancing an electrical bridge circuit including the first photo-electric device and adjustable optical wedge compensating means is provided for varying the amount of light allowed to impinge on the cell containing the suspension.

A symmetrical pair of current amplifiers may be connected with the output circuits of the two photo-electric cells and adapted to operate as a balanced circuit with a microammeter as a null indicator.

Alternatively the apparatus may include a reflecting surface for reflecting the transmitted emergent light on to a photo-electric device, means for collecting the forwardly scattered light and directing the same on to a second photo-electric device and adjustable optical wedge compensating means for varying the amount of light allowed to impinge on the first photo-electric device whereby the ratio of the forwardly scattered light from the suspension to the directly transmitted light and hence the concentration of the suspension can be determined.

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A shunt path of light as hereinafter described may be provided from the light source to the cell containing the suspension together with an adjustable stop adapted to balance out stray scattered light, for example, from surface reflections, not arising from suspended matter in the suspension.

In order that the invention may be readily understood, the following description is given, by way of example, of one arrangement of an apparatus for measuring the concentration of bacterial suspensions.

In the accompanying drawings:—

Figure 1 is a diagrammatic arrangement of the apparatus;

Figure 2 shows an arrangement of apparatus to allow for stray scattered light from surface reflections; and

Figure 3 shows a simplified form of nephelometer construction.

A light source 1 comprising a tungsten lamp having a straight helical filament is positioned between a vacuum photo cell 2 and a standard comparison photocell 3. A filtering unit 4, a diffusing screen 5 and an iris diaphragm 6 are placed in the light path between the light source 1 and the photocell 3 and a fine threaded thumbscrew 7 is positioned with its pointed end 8 in the light path between the said filtering unit and the diffusing screen. Plano convex lens combinations 9 and 10 are placed in the light path between the light source 1 and the photocell 2, and a diaphragm 11, situated between these lens combinations, is provided with a rectangular slit 12. An annular optical wedge 13 having a linear law of optical density is mounted in a graduated rotatable drum (not shown) and is positioned between the lens combination 9 and the diaphragm 11. A slide 14 has near one of its sides a convex lens 15 and an opaque stop 16 aligned centrally in front of the lens, and near its other side the slide carries an adjustable slit 17. A filter holder 18 is positioned between the component plano convex lenses of the lens combination 9 and an optical cell 19 containing the specimen is positioned between the lenses of the combination 10. The lenses of the combination 9 and 10 are all alike and are bloomed on all faces. Lastly a variable compensating stop 20 is located between the slide 14 and the photocell 2 and the two photocells 2 and 3 are each connected to a direct-coupled current amplifier (not shown) connected to a common centre zero microammeter (not shown).

When the apparatus is in operation the photocell 3 receives light from the lamp 1 through the filtering unit 4, diffusing screen 5 and iris diaphragm 6 whilst the photocell 2 simultaneously receives light from the same source after the light has traversed the

lens combination 9, the optical wedge 13, the slit 12, the cell 19, the lens combination 10, the slide 14 and variable compensating stop 20. The lens combination 9 forms an image of the lamp filament on the slit 12 and the second lens combination 10 receives the light from the slit and brings it to a focus. The slide 14 is positioned so that the opaque stop 16 which is a little larger than the image of the slit is in alignment with the optical axis of the system and the light from the slit is brought to a focus near the stop by the lens combination 10. The said stop 16 prevents the direct image forming light from reaching the photocell 2 and the convex lens 15 collects the scattered light only. When the slide 14 is moved across so that the adjustable slit 17 is placed in the position previously occupied by the opaque stop 16, and so aligned with the optical axis of the system, the photocell 2 receives only direct light. The outputs from the photocells 2 and 3 are fed into the symmetrical pair of direct coupled current amplifiers which work into a common centre zero microammeter used as a null indicator. It is desirable that the detecting amplifiers should be approximately balanced when no light falls on the photocells. When the wedge 13 is set at its position of minimum density and the slide 14 is set so that only scattered light reaches the photocell 2 then the outputs of the photocells 2 and 3 can be made equal by adjusting the iris diaphragm 6. Now when the slide 14 is moved across to its alternative position so that the slit 17 is placed into alignment with the optical axis of the system the amount of light falling on the photocell 2 increases and a new balance is obtained by rotating the annular wedge 13 until the microammeter reading is again zero. The wedge reading is then a measure of the concentration of the sample.

When using a very dense suspension it may not be possible to obtain a balance by rotating the annular wedge 13, i.e. the light scattered by the sample is more intense than the transmitted light. In this case the first balance, with the iris diaphragm 6, is carried out again with the split 17 in the light path, and the second with the wedge, after replacing the split 17 by the opaque stop 16. Then if  $m$  is the maximum reading of the wedge, and  $x$  the reading obtained, the sample concentration is measured by  $2m - x$ . In this way the wedge can be used twice, over and its effective range of density, and with a wedge of moderate density gradient a fairly open scale is obtained.

An important advantage of the above arrangement is that the range of light intensity which the photocells are required to accept is much less than the range of sample concentrations so that no special precau-

tions need be taken in the choice of cells.

Stray scattered light from surface reflections and dirt on the lenses would cause a positive reading when there was no specimen in the light path thus interfering with the relationship between the wedge reading and the concentration of the sample and reducing the sensitivity of the instrument when dealing with dilute suspensions. This defect can largely be remedied by blooming the lenses and by providing a shunt light path, now to be described, between the lamp 1 and the slit 12. Referring now to Figure 2, small mirrors 21 and 22 are used to direct the by-passing light through one end of the slit 12 and the filter 18 is arranged to intersect both the main and shunt light paths. An adjustable stop 23 is used to vary the amount of light reaching the slit 12 by the shunt path and it can be shown that if the amount is adjusted to the value  $p/(1-p)$ , where  $p$  is a fraction representing the amount of stray scattered light not arising from suspended matter in the specimen, the relationship between the wedge reading and the concentration of the sample is restored.

Because the scattered light is bluer than the transmitted, a change in the spectral sensitivity of the photocells might appreciably alter the response of the instrument consequently the variable stop 20 which occludes part of the scattered light, is fitted to compensate for such a change.

The instrument may be made relatively insensitive to changes of colour by placing a yellow filter in the light path.

The wedge reading is a linear function of the logarithm of the concentration in the first part of the concentration range, i.e. wedge readings 0—20. In the part of the concentration range giving wedge readings 25—38 the law is again linear but the slope is approximately doubled because of considerable secondary scatter and the readings in this range approximate to a linear function of the logarithm of the square of the concentration.

To compensate for stray scattered light the cell 19 is filled with the clear liquid which is to be used as a suspending medium, the wedge is set at reading 25 whilst the slide 14 is positioned so that the stop 16 is in alignment with the optical axis of the system and the filter holder 18 is removed. The micrometer reading is brought to zero by adjusting the iris 6 and screw 7. A metal blank (not shown) is inserted in place of the filter holder 18 so as to screen the main light path but leave the shunt path open. The slide is then adjusted so as to place the adjustable slit 17 in alignment and the microammeter reading is returned to zero by adjusting the stop 23.

To ascertain the measure of concentration of a suspension the specimen cell is filled

by suction with the suspension, the wedge is set at graduation 25 and the slide 14 adjusted to bring the stop 16 in alignment with the optical axis of the system. The microammeter reading is set to zero by adjusting the iris diaphragm 6 and the screw 7. The slide is then adjusted to place the slit 17 into alignment and the wedge is again turned until a balance is reached.

If no balance is thus found by turning the wedge, it is returned to graduation 25 and without altering the slide, the zero reading is again obtained using the iris 6 and the screw 7. The slide is now moved to its alternative position and the wedge turned until balance is reached. A measure of the concentration of the suspension is the difference obtained by subtracting the wedge reading from 50.

In the simplified nephelometer construction shown at Figure 3 the transmitted and scattered light are compared simultaneously. An image of a slit 12a is formed and reflected by a mirror 24 through the wedge 13a on to the photocell 3a. Simultaneously the scattered light is collected by the lens 15a and directed on to the photocell 2a so that a balance can be obtained simply by adjusting the wedge 13a.

The wedge could be servo-driven from the detecting amplifiers and a continuous record could be provided of concentration changes in the specimen. In this way the instrument may be used to advantage in connection with continuous culture techniques.

Apart from its general convenience, the instrument is specially suited to the handling of pathogens, because only very concentrated suspensions ( $>10^{10}$ /cc) need to be diluted before measurement. Advantage may be taken of this feature by using a special form of cell of the "flow through" type having a top limb connected to a 3-way tap and a fine capillary tube to a vacuum pump and its bottom limb connected to a reservoir containing the suspension to be measured. Thus the cell can be gently filled from and emptied into a reservoir connected to the lower limb or can be conveniently washed out with cleaning agents.

What we claim is:—

1. An apparatus for measuring the concentration of suspension of particles in a liquid medium by comparing the light scattered by the suspension with the light transmitted by the same, comprising a light source, a cell for containing the suspension, a lens system for focusing the transmitted emergent light, means associated with the said lens system adapted to be adjusted at will either to cut off the directly transmitted emergent light and to collect the forwardly-scattered light from the suspension and direct the same on to a photo-electric device

or to cut off the forwardly-scattered light and to direct the said transmitted light on to the said photo-electric device whereby the ratio of the forwardly-scattered light from the said suspension to the directly transmitted light and hence the concentration of the suspension can be determined.

2. An apparatus for measuring the concentration of a suspension of particles in a liquid medium by comparing the light scattered by the suspension with the light simultaneously transmitted by the same, comprising a light source, a cell for containing the suspension, a lens system for focusing the transmitted emergent light, a photo-electric device for receiving the light transmitted through the suspension, a second photo-electric device associated with the same light source for balancing an electrical bridge circuit including the first photo-electric device, means associated with the said lens system adapted to be adjusted at will either to cut off the directly transmitted emergent light and to collect the forwardly-scattered light from the suspension and to direct the same on to the first photo-electric device, or to cut off the forwardly-scattered light and to direct the said transmitted light on to the first photo-electric device, and adjustable optical wedge compensating means for varying the amount of light allowed to impinge on the cell containing the suspension; whereby the ratio of the forwardly-scattered light from the suspension to the directly transmitted light and hence the concentration of the suspension can be determined.

3. An apparatus for measuring the concentration of a suspension of particles in a liquid medium by comparing the light scattered forwardly by the suspension with the light directly transmitted by the same, comprising a light source, a cell for con-

taining the suspension, a lens system for focusing the transmitted emergent light, a reflecting surface for reflecting the said transmitted light on to a photo-electric device, means for collecting the forwardly scattered light and directing the same on a second photo-electric device and adjustable optical wedge compensating means for varying the amount of light allowed to impinge on the first photo-electric device whereby the ratio of the forwardly-scattered light from the suspension to the directly transmitted light and hence the concentration of the suspension can be determined.

4. Apparatus as claimed in Claim 1 or 2, wherein a shunt path of light from the light source to the cell containing the suspension is provided together with an adjustable stop adapted to balance out stray scattered light, for example from surface reflections, not arising from suspended matter in the suspension.

5. Apparatus according to Claim 2, 3 or 4 in combination with a symmetrical pair of current amplifiers connected with the output circuits of the two photo-electric cells and arranged to operate as a balanced circuit with a microammeter as a null indicator.

6. Apparatus for measuring the concentration of a suspension of particles in a liquid medium substantially as described with reference to Figures 1 and 2, of the accompanying drawings.

7. Apparatus for measuring the concentration of particles in a liquid medium substantially as described with reference to Figure 3 of the accompanying drawings.

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#### PROVISIONAL SPECIFICATION.

#### Improvements in or relating to Photometric Apparatus.

We, NATIONAL RESEARCH DEVELOPMENT CORPORATION, a British Corporation established by Statute, of 1 Tilney Street, London, W.1, do hereby declare this invention to be described in the following statement:—

This invention relates to photometric apparatus, more particularly to turbidometers or nephelometers for use in measuring the concentration of suspensions of particles in a liquid medium.

The object of the invention is to provide an improved apparatus for use with suspensions having a wide range of concentrations especially bacterial suspensions of any concentration normally encountered and which

will permit the concentration to be measured directly without the necessity of tedious and hazardous preparation of suitable dilutions.

The apparatus is of the kind in which a beam of light is passed through a cell of suitable thickness with parallel sides containing the suspension. During the passage of the light through the suspension some of the light is scattered by the suspended particles. In a bacterial suspension, the particle size is such that most of this light is scattered in a direction close to that of the primary beam. It can be shown that the ratio of the intensity of the scattered light

to the intensity of the transmitted light is proportional to the concentration. By a comparison of the light scattered by a suspension with the light simultaneously transmitted by the same, it is possible to obtain a measure of the concentration over a wide range.

According to the present invention, apparatus for measuring the concentration of a suspension of particles in a liquid medium by comparing the light scattered by the suspension with light simultaneously transmitted by the same, comprises a light source, a cell for containing the suspension, a lens system for focusing the transmitted light, means associated with said lens system adapted to be adjusted at will either to cut off the directly transmitted light received and to collect the forward-scattered light from the cell and direct the same on to a photo-electric cell, or to cut off the forward scattered light and direct the said transmitted light on to the photo-electric cell, and adjustable optical wedge compensating means for varying the intensity of light allowed to impinge on the cell containing the suspension, whereby the ratio of the forward-scattered light from the cell to the directly transmitted light, and hence the concentration of the suspension, can be determined. Preferably a second photo-electric cell is associated with the light source and adapted to provide a compensating source of electric current for balancing the current produced by the transmitted or scattered light incident on the first-mentioned photo-electric cell. A symmetrical pair of current amplifiers may be connected with the output circuits of the two photo-electric cells and adapted to operate as a balanced circuit with a microammeter as a null indicator.

In order that the invention may be readily understood, the following description is given by way of example of one arrangement of an apparatus for measuring the concentration of bacterial suspensions.

The light source is a tungsten lamp with a straight helical filament. There are two light paths from the lamp terminating in vacuum photocells  $P_1$ ,  $P_2$ .  $P_1$  is a comparison standard used to make the electronic detector circuit symmetrical and to compensate for changes in intensity of illumination. It receives light through filters (neutral and heat-absorbing glasses), a diffusing screen, and an iris. Light in the other path passes through two plano-convex achromats which form an image of the filament on a slit about 1 mm wide by 5 mm long. An optical wedge is arranged in front of the slit and is actually annular in form and is mounted in a graduated drum. A second pair of lenses receive the light from the slit and bring it to a focus. Between these lenses the

light is parallel, and passes through the cell containing the specimen, and is brought to the said focus on an opaque stop—a blackened trough of copper foil—a little larger than the image of the slit. Behind the stop is a lens which focuses the last surface of the second pair on the photo-cell  $P_2$ . The stop is mounted in a slide  $X$  which carries also an adjustable slit  $S_2$ . Thus with  $X$  in the one position ("C position") the stop prevents the direct, image-forming, light from reaching  $P_2$ , and the lens collects the scattered light only. When the slide is moved across into its alternative position (" $S_2$  position") the slit falls into the place previously occupied by the stop, and allows  $P_2$  to receive only the direct light. The device of stopping out the direct light and collecting the intense forward-scattered light is a very useful one and is adopted in some existing instruments, e.g. Libby, 1938; Silverman, 1941; Sowerby & Walton, 1945. The forward-scattered light has about 10 times the intensity of that scattered at  $90^\circ$  to the primary beam.

The outputs from the photo-cells  $P_1$ ,  $P_2$  are fed into a symmetrical pair of direct-coupled current amplifiers working into a centre-zero microammeter used as a null indicator only.

Now suppose that the wedge is set at its position of minimum density and the slide  $X$  is set so that only scattered light reaches  $P_2$ . The outputs of  $P_1$  and  $P_2$  can be made equal by adjusting the iris in front of  $P_1$ .

If then the slide  $X$  is moved into its alternative position, the light falling on  $P_2$  will increase, and a new balance can be obtained by turning the wedge until the indicator reads zero again. The wedge reading is then a measure of the concentration of the sample. It may happen with very dense suspension that no balance can be obtained with the wedge, i.e. the scattered light is more intense than the transmitted. In this case the first balance, with the iris, is carried out again with the slide  $X$  in its alternative position with the slit  $S_2$  in the light path, and the second, with the wedge, after replacing  $S_2$  by the stop with the slide  $X$  in the "C position". Then if  $m$  is the maximum reading of the wedge, and  $x$  the reading obtained, the sample concentration is measured by  $2m - x$ . The wedge can in this way be used twice over, and its effective range of density doubled. A fairly open scale is obtained with a wedge of moderate density gradient.

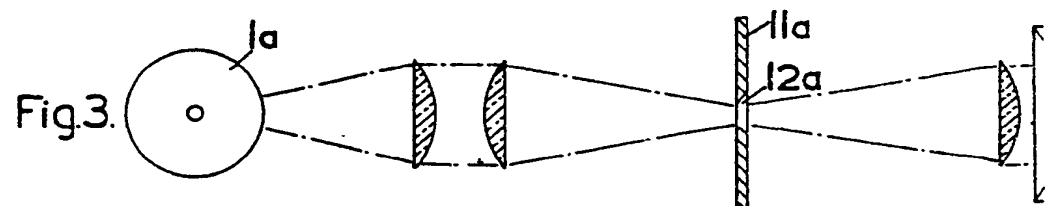
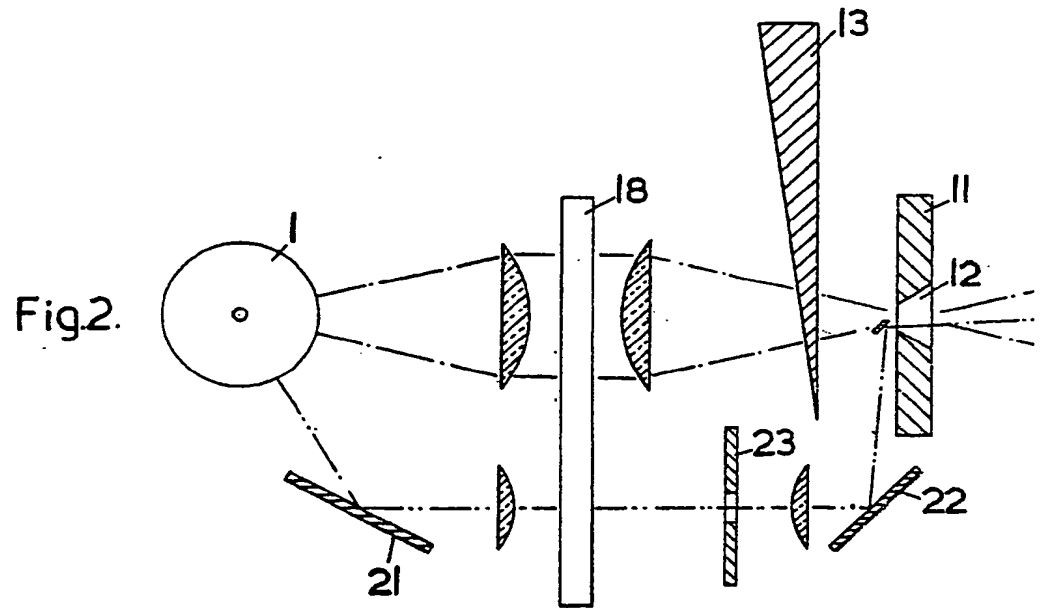
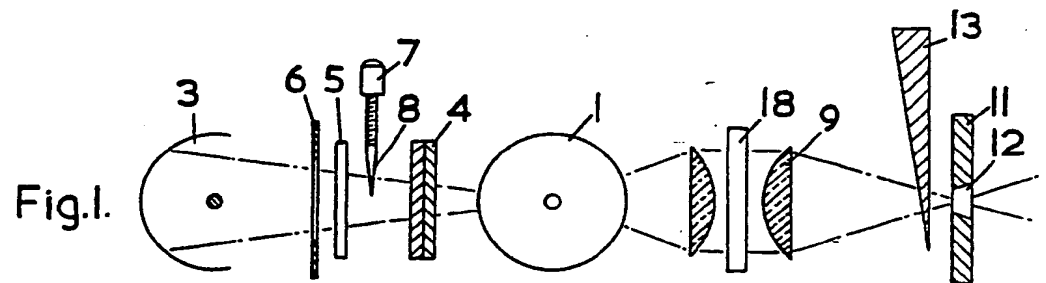
It is an important advantage of the arrangement used that the range of light intensity which the photocells are required to accept is much less than the range of sample concentrations. Thus no special precautions need be taken in the choice of cells.

In order to compensate for stray scattered

light from surface reflections and other causes, which may give a positive reading with no specimen in the light path and reduce the sensitivity with dilute solutions, a shunt path for light from the lamp to the first slit may be provided by mirrors and an adjustable stop, so that a very small mirror directs the by-passed light through one end of the slit. The adjustable stop may be used to balance out any scattered light not arising from suspended matter in the specimen. 10

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# 755,900 COMPLETE SPECIFICATION

1 SHEET

*This drawing is a reproduction of the Original on a reduced scale.*

